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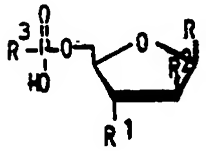
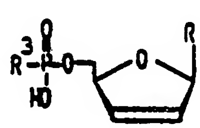
(54) Title: 5'-HYDROGENPHOSPHONATES AND 5'-METHYLPHOSPHONATES OF SUGAR MODIFIED NUCLEOSIDES, COMPOSITIONS AND USES THEREOF


(I)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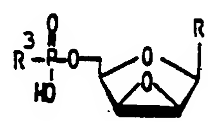
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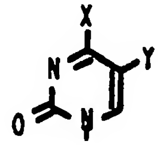
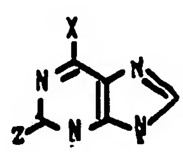

(a)


(b)

(57) Abstract

The present invention concerns compounds having one of the structures (I), (II), (III), wherein R is (a) or (b), R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, R^3 is hydrogen or an alkyl group of one to four carbons, X is a hydroxy, a thiol, or an amino group, Y is hydrogen, a halogen or an alkyl group of one to four carbons, and Z is a hydrogen, hydroxy, or an amino group. The present invention also provides pharmaceutical compositions comprising a pharmaceutically effective amount of a compound according to the subject invention and a pharmaceutically acceptable carrier. Finally, the invention provides methods to suppress viral infection.

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5'-Hydrogenphosphonates and 5'-Methylphosphonates of Sugar Modified Nucleosides, Compositions and Uses Thereof

The invention described herein was made in the course of work under Grant Nos. CA-08748 and AI-26056 from the National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services.

Background Of The Invention

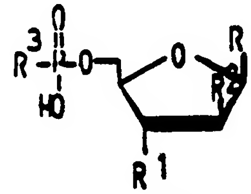
The only clinically available agent for the treatment of acquired immune deficiency syndrome (AIDS) in the United States is 3'-azido-3'-deoxythymidine (AZT). [Mitsuya et al., Proc. Nat Acad. Sci., USA, 1985 82, 7096] Several 2',3'-dideoxynucleosides are also reported [Mitsuya et al., Proc. Nat. Acad. Sci., USA, 1986, 83, 1911] as active against human immune deficiency virus (HIV), the responsible pathogen that causes AIDS. These nucleosides are converted into their corresponding 5'-mono-nucleotides by the action of cellular nucleoside kinase(s) followed by stepwise phosphorylation catalyzed by cellular nucleotide kinases to their corresponding 5'-triphosphates. These nucleoside 5'-triphosphates inhibit proviral DNA synthesis catalyzed by HIV reverse transcriptase (RT) by incorporation to the 3' position of the growing DNA terminal.

Many nucleosides are poor substrates for deoxynucleoside kinase(s) due to rather restricted structural requirement of the enzyme(s). Conversion of the 5'-monophosphate of these nucleosides into their corresponding 5'-triphosphates usually occurs readily in the cell. Nucleoside-5'-monophosphates cannot be used for treatment of AIDS, because they can hardly penetrate the cell membrane due to strong acidic nature. Nucleoside-5'-hydrogenphosphonates, weak acidic compounds, however, may penetrate cell membrane and

may be oxidized to their corresponding phosphates and then further converted into the corresponding triphosphates in the cell, or the 5'-hydrogen-phosphonates may serve as substrates for nucleotide kinases forming the triphosphate analogues, pyrophosphorylhydrogenphosphonates, which then inhibit the viral DNA synthesis catalyzed by the reverse transcriptase.

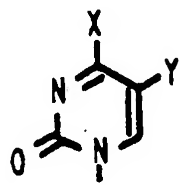
Summary Of The Invention

The present invention provides a compound having the structure:

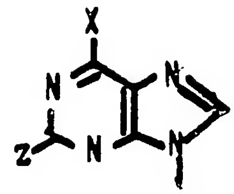


I

wherein R is



or



,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

R^2 is hydrogen, halogen, an azido, an amine or an alkyl group of one to four carbons,

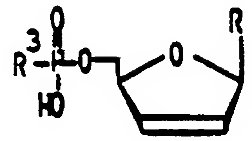
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

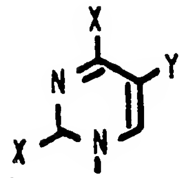
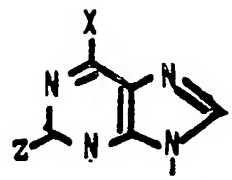
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

Z is a hydrogen, a hydroxy, or an amino group.

The present invention also provides a compound having the structure:



II

wherein R  or  ,

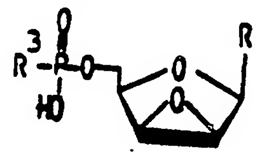
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

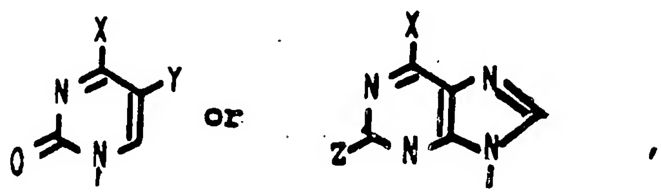
Z is a hydrogen, a hydroxy, or an amino group.

Further, the present invention provides a compound having the structure:



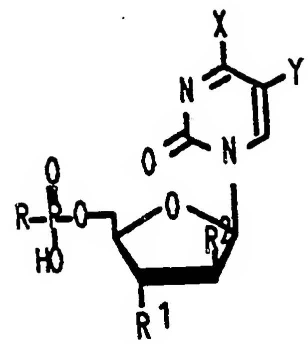
III

wherein R



R³ is hydrogen or an alkyl group of one to four carbons,
X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and
Z is a hydrogen, a hydroxy, or an amino group.

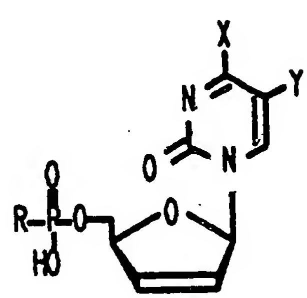
The present invention provides a compound having the structure:



I

X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

Also, the invention provides a compound having the structure:

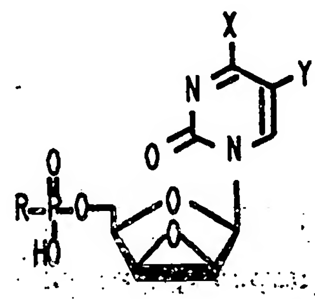


II

X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

Further, the invention provides a compound having the structure:

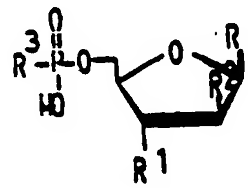


X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

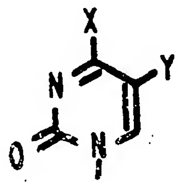
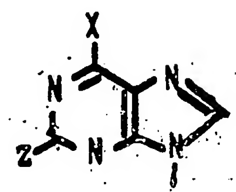
The present invention also provides pharmaceutical compositions comprising a pharmaceutically effective amount of a compound according to the subject invention and a pharmaceutically acceptable carrier. Finally, the invention provides methods for treating viral infections.

Detailed Description Of The Invention

The present invention provides a compound having the structure:



I

wherein R is  or 

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

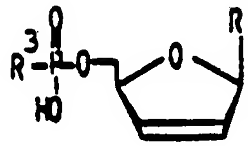
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

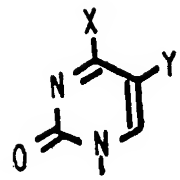
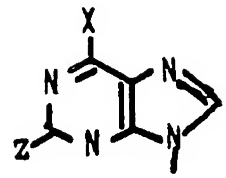
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

Z is a hydrogen, a hydroxy, or an amino group.

The present invention also provides a compound having the structure:

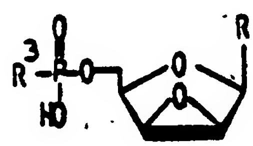


II

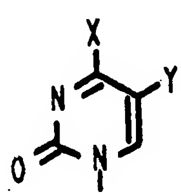
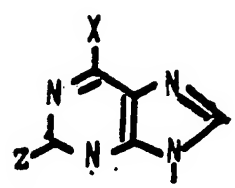
wherein R is  or  ,

R^3 is hydrogen or an alkyl group of one to four carbons,
X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and
Z is a hydrogen, a hydroxy, or an amino group.

Further, the present invention provides a compound having the structure:



III

wherein R is  or  ,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an

alkyl group of one to four carbons,

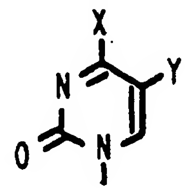
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

Z is a hydrogen, hydroxy, or an amino group.

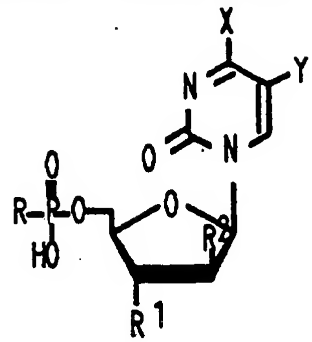
The present invention also provides a compound of structures I, II or III wherein R is:



wherein X is a hydroxy, a thiol, or an amino group,

Y is hydrogen, halogen or an alkyl group of one to four carbons.

The present invention provides a compound having the structure:



I

X is a hydroxy, a thiol, or an amino group,

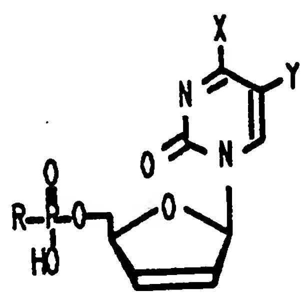
Y is hydrogen, a halogen or an alkyl group of one to four carbons,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and

R is hydrogen or an alkyl group of one to four carbons.

Also, the invention provides a compound having the structure:



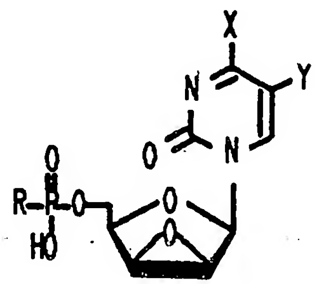
II

X is a hydroxy, a thiol, or an amino group,

Y is hydrogen, a halogen or an alkyl group of one to four carbons,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

Further, the invention provides a compound having the structure:



X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

Compounds of structures I, II or III may be used to suppress viral replication and treat infection.

The following compounds are examples of compounds useful in accordance with the present invention:

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-β-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) uracil,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl)-5-fluorouracil.

The following compounds are still further examples of

compounds of the present invention:

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) thymine,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) uracil.

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-threo-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-threo-

pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D-threo-pentofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methyl-phosphonyl-ß-D-arabinofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methyl-phosphonyl-ß-D-arabinofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methyl-phosphonyl-ß-D-arabinofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methyl-phosphonyl-ß-D-arabinofuranosyl)-5-fluorouracil.

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methyl-phosphonyl-ß-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methyl-phosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methyl-phosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) thymine,

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) uracil.

The subject invention also provides a pharmaceutical composition which comprises a pharmaceutically effective amount of a compound of structures I, II or III or a pharmaceutically acceptable metal addition salt thereof and a pharmaceutically acceptable carrier.

The term "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers such as sterile solutions, tablets, coated tablets and capsules. Typically such carriers, contain excipients such as starch, milk, sugar, certain types of clay, gelatin, steric acid, talc, vegetable fats or oils, gums, glycols, or other known

excipients. Such carriers may also include flavor and color additives or other ingredients. Compositions comprising such carriers are formulated by well known conventional methods. However, the compositions comprising the compound of structures I, II or III or a metal salt thereof, are previously unknown.

This invention further concerns a method of treating a viral infection so as to render the infection incapable of viral replication which comprises contacting the viral infection with an effective amount of a compound of structure I, II or III.

The amount of the compound required will vary considerably depending upon conditions. However, these amounts are readily determinable by one skilled in the art.

Additionally, this invention provides a method of treating a viral infection which comprises contacting the viral infection with an effective amount of the pharmaceutical composition described above, i.e. 1 to 200 mg/kg of body weight of a subject.

This invention also provides a method of treating a subject which comprises administering to the subject an effective amount of the pharmaceutical composition described above.

In this method, the administration of the compound may be effected by any of the well known methods, including but not limited to oral, intravenous, intramuscular, and subcutaneous. The method of delivery, the amount to be and the frequency of delivery, are expected to vary according to the situation, the carrier used, and result desired. However, those variables are readily determinable by one skilled in the art.

The term "subject" includes but is not limited to domestic animals and human beings.

This invention further provides a method of treating a subject having a viral infection which comprises administering to the subject an effective amount of the compound to suppress the viral replication. A subject may be any warm-blooded animal, preferably human. The viral infection may be any viral infection, including but not limited to human immunodeficiency virus, hepititis virus or cytomegalo virus.

The following Experimental Detail Section and Examples are set forth to aid in an understanding of the invention. These sections are not intended to, and should not be construed to, limit in any way the invention set forth in the claims which follow thereafter.

Experimental Details

SYNTHESIS OF NUCLEOSIDE-5'-HYDROGENPHOSPHONATES. To a solution of nucleoside (0.2 mmol) in a solvent (2 mL) are added 0.6 M solution of phosphorous acid tri-n-butylammonium salt in pyridine (0.5 mL) and N,N'-dicyclohexyl-carbodiimide (0.6 mmol). The mixture is stirred at room temperature. The reaction is monitored by thin layer chromatography on a silica gel plate using isopropanol : 25% ammonium hydroxide : water (7:1:2 v/v) (system 1) as the solvent. After all the nucleoside is consumed, the mixture is centrifuged for 10 minutes. The supernatant is removed by decantation. The solid is twice washed with water. The product is isolated by preparative layer chromatography on a silica gel plates using system 1.

SYNTHESIS OF NUCLEOSIDE-5'-METHYLPHOSPHONATES. To a solution of nucleoside in a solvent mixture are added at 0 °C successively dichloro-methylphosphoryl oxide and 1,2,4-tetrazole. The mixture is stirred at 0 °C for 1 hour and then at room temperature. After completion of the reaction as judged by thin layer chromatography on a silica gel plate in system 1, the mixture is cooled to 0 °C, and the reaction is quenched by addition of triethylamine and water. The mixture is stirred for 2 hours at 4 °C, and then is concentrated in vacuo. The nucleoside-5'-methyl-phosphonates are isolated by preparative layer chromatography on silica gel plates using system 1.

The following examples are illustrated of the process and products of the present invention, but are not to be construed as limiting.

Example 1

To a solution 3'-azido-3'-deoxythymidine (53 mg, 0.2 mmol) in pyridine (2 mL) are added 0.6 M solution of phosphorous acid tri-n-butylammonium salt in pyridine (0.5 mL) and N,N'-dicyclohexyl-carbodiimide (125 mg, 0.6 mmol). The mixture is stirred for 4 hours at room temperature, and then is centrifuged for 10 minutes. The supernatant is removed by decantation. The solid is twice washed by dispersion in water (1 mL each) followed by centrifugation. The combined supernatants are concentrated to dryness in vacuo. The residue is dissolved in a minimal amount of pyridine and applied to a silica gel plate (20 x 20 x 0.15 cm), and the plate is developed in system 1. The UV absorbing band corresponding to the nucleoside-5'-phosphonate is scraped, and then extracted with system 1 (30 mL). The solvent is removed by evaporation in vacuo, and the residue is reevaporated with water (2 mL). The residue is dried azeotropically by evaporation with ethanol (2 mL x 2) in vacuo. 1-(3-azido-3-deoxy-5-O-hydrogen-phosphonyl-β-D-*erythro*-pentofuranosyl)thymine (58 mg, 84% yield) is obtained as colorless foam.

By following the same procedure but using the corresponding nucleosides instead of 3'-azido-3'-deoxythymidine, the following nucleoside-5'-hydrogenphosphonates are prepared:

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-β-D-*glycero*-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-β-D-*glycero*-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-β-D-*glycero*-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-O-hydrogenphosphonyl-β-D-lyxofuranosyl)-5-fluorouracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-β-D-lyxofuranosyl)-5-

fluorouracil,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) uracil,

1-(2,3-Dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-threo-pentofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl)-5-fluorouracil.

Example 2

To a solution of 1-(2,3-Dideoxy-2,3-didehydro-ß-D-glycero-pentofuranosyl) thymine (45 mg, 0.2 mmol) in trimethylphosphate (2 mL)) are added 0.6 M solution of phosphorous acid tri-n-butyl-ammonium salt in pyridine (0.5 mL) and N,N'-dicyclohexyl carbodimide (125 mg, 0.6 mmol). The mixture is stirred for 8 hours at room temperature, and

then is centrifuged for 10 minutes. The supernatant is removed by decantation. The solid is twice washed by dispersion in water (1 mL each) followed by centrifugation. The combined supernatants are concentrated to dryness _in vacuo_. The residue is dissolved in a minimal amount of pyridine and applied to a silica gel plate (20 x 20 x 0.15 cm), and the plate is developed in system 1. The UV absorbing band corresponding to the nucleoside-5'-phosphonate is scraped, and then extracted with system 1 (30 mL). The solvent is removed by evaporation in vacuo, and the residue is reevaporated with water (2 mL). The residue is dried azeotropically be evaporation with ethanol (2 mL x 2) in vacuo. 1-(2,3-Dideoxy-2,3-didehydro-5-O-hydrogenphosphonyl-ß-D-_glycero_-pentofuranosyl) thymine (25 mg, 42% yield) is obtained as colorless foam.

By following the same procedure but using the corresponding nucleosides instead of 1-(2,3,-Dideoxy-2,3-didehydro-ß-D-_glycero_-pentofuranosyl)thymine, the following nucleoside-5'-hydrogen phosphonates were prepared:

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-_glycero_-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-_glycero_-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-_glycero_-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) thymine, and

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) uracil.

Table 1 lists the reaction conditions, yields and chromatogaphic and UV characteristics for some representative nucleoside-5'-hydrogen-phosphonates.

Table 3 lists the ^{1}H NMR parameters for some representative nucleoside-5'-hydrogenphosphonates.

Table 5 lists the ^{32}P NMR data for some representative nucleoside-5'-phosphonates

Example 3

To a solution of 1-(2,3 dideoxy-ß-D-glycero-pentofuranosyl) cytosine (43 mg, 0.2 mmol) in trimethylphosphate (2.0 mL) are added at 0 °C successively dichloromethylphosphoryl oxide (80 mg, 0.6 mmol) and 1,2,4-tetrazole (20 mg). The mixture is stirred at 0 °C for 1 hour and then at room temperature for 4 hours. The mixture is cooled to 0 °C, and the reaction is quenched by addition of triethylamine (0.2 mL) and water (0.2 mL). The mixtrue is stirred for 2 hours at 4 °C, and then is concentrated in vacuo. 1-(2,3-dideoxy-5-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) cytosine is isolated by preparative layer chromatography on a silica gel plate as Example 1 (29 mg, 42% yield, as a colorless foam).

By following the same procedure but using the corresponding nucleosides instead of 1-(2',3'-dideoxy-B-D-glycero-pentofuranosyl)cytosine, the following nucleoside-5'-methylphosphonates are prepared:

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-

lyxofuranosyl)-5-fluorouracil,
1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) thymine,
1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) uracil,
1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) cytosine,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) thymine,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) uracil,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-erythro-pentofuranosyl) cytosine,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-threo-pentofuranosyl) thymine,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-threo-pentofuranosyl) uracil,
1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-β-D-threo-pentofuranosyl) cytosine,
1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-β-D-arabinofuranosyl) thymine,
1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-β-D-arabinofuranosyl) uracil,
1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-β-D-arabinofuranosyl) cytosine, and
1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-β-D-arabinofuranosyl)-5-fluorouracil.

Example 4

To a solution of 1-(2,3-anhydro-β-D-lyxopentofuranosyl) cytosine (45 mg, 0.2 mmol) in trimethylphosphate (1.0mL) are added at 0°C successively dichloromethyl phosphoryl oxide

(80 mg, 0.6 mmol) and 1,2,4-tetrazole (20 mg). The mixture is stirred at 0 °C for 1 hour and then at room temperature for 14 hours. The mixture is cooled to 0 °C, and the reaction is quenched by addition of triethylamine (0.2 mL) and water (0.2 mL). The mixture is stirred for 2 hours at 4 °C, and then is concentrated in vacuo. 1-(2,3-Anhydro-5-O-methylphosphonyl-β-D-lyxofuranosyl) cytosine is isolated by preparative layer chromatography on a silica gel plate as Example 1 (28 mg, 46% yield, as a colorless foam).

By following the same procedure but using the corresponding nucleosides instead of 1-(2,3-anhydro-β-D-lyxopentofuranosyl)cytosine, the following nucleoside-5'-methylphosphonates are prepared:

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-β-D-<u>glycero</u>-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-β-D-<u>glycero</u>-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-β-D-<u>glycero</u>-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-methylphosphonyl-β-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-methylphosphonyl-β-D-lyxofuranosyl) thymine, and

1-(2,3-Anhydro-5'-O-methylphosphonyl-β-D-lyxofuranosyl) uracil.

Table 2 lists the reaction conditions, yields and chromatographic and UV characteristics of some of these nucleoside-5'-methyl-phosphonates that are synthesized by the above procedure.

Table 4 lists the ^{1}H NMR parameters for some of these

nucleoside-5'-methylphosphonates.

Table 1. Experimental conditions for the synthesis of 5'-hydrogenphosphonates (I, R=H)

X	Y	R^1	R^2	solvent (mL)	time (hrs)	yield (%)	chromatography solvent 1 Rf	chromatography solvent 2 Rf	UV absorption in H^2O (nm) max	min	pH
O	Me	H	F	pyridine (2)	4	84	0.88	0.80	266	234	7.0
O	Me	N_3	F	pyridine (2)	8	76	0.80	0.80	266	234	7.0
O	Me	N_3	H	pyridine (2)	12	64	0.68	0.55	266	234	7.0
O	Me	H	H	MeCN (2)*1	38	61	0.70	0.66	268	234	7.0
O	Me	F	H	pyridine (2)	6	78	0.62	0.56	266	234	7.0
O	H	H	H	MeCN (2)*2	8*3	36	0.54	0.48	270	247	7.0
NH_2	H	H	H	pyridine (2)	12	47	0.76	0.74	261	230	7.0

Table 1 (cont'd)

X	Y	R^1	R^2	solvent (mL)	time (hrs)	yield (%)	chromatography solvent 1 Rf	chromatography solvent 2 Rf	UV absorption in H^2O (nm) max	UV absorption in H^2O (nm) min	pH
Experimental conditions for the synthesis of 5'-hydrogenphosphonates (II, R = H)											
NH_2	H			$(MeO)_3PO$ (2)	4*3	36	0.73	0.73	270	247	7.0
									277	238	1.0
O	Me			$(MeO)_3PO$ (2)	8*3	40	0.63	0.60	266	234	7.0
O	H			MeCN (1)/$(MeO)_3PO$(0.5)	4	52	0.54	0.53	261	230	7.0
Experimental conditions for the synthesis of 5'-hydrogenphosphonates (III, R = H)											
O	H			$(MeO)_3PO$ (0.5)	12	52	0.43	0.54	261	230	7.0
O	F			$(MeO)_3PO$ (0.5)	12	58	0.45	0.45	268	234	7.0
NH_2	H			$(MeO)_3PO$ (1)	14	46	0.37	0.34	270	247	7.0
									277	238	1.0

*1 with 0.4 mL of N-methylimidazole

*2 with 0.5 mL of $(MeO)_3PO$

*3 beyond this time, side reactions take place

Table 2. Experimental conditions for the synthesis of 5'-methylphosphonates (I, R = Me)

X	Y	R^1	R^2	$(MeO)_3PO$ (mL)	MeCN (mL)	time (hr) 0°C	time (hr) rt*	yield (%)	chromatography solvent (1)	chromatography solvent (2)	UV absorption in H^2O (nm) max	min	pH
0	H	H	H	0.5	2	14	4	54	0.73	0.70	261	232	7.0
0	Me	H	H	2	0	1	4	86	0.53	0.50	266	234	7.0
NH_2	H	H	H	1.5	0	18	0	47	0.56	0.52	270	247	7.0
0	Me	F	H	0.2	2	14	6	64	0.73	0.70	277 261	238 232	1.0 7.0
0	Me	N_3	H	0.3	1	6	2	78	0.85	0.75	266	234	7.0
0	Me	H	F	0.3	1	6	8	57	0.65	0.54	265	234	7.0
0	Me	N_3	F	0.3	2	14	0	58	0.87	0.75	265	235	7.0

Table 2 (cont'd)

X	Y	R^1	R^2	$(MeO)_3PO$ (mL)	MeCN (mL)	time (hr) 0°C	time (hr) rt*	yield (%)	chromatography solvent (1)	chromatography solvent (2)	UV absorption in H^2O (nm) max	min	pH
Experimental conditions for the synthesis of 5'-methylphosphonates (II, r = Me)													
NH_2	H			2	0	1	4	42	0.71	0.68	270	247	7.0
											277	238	1.0
0	H			0.5	0	14	0	67	0.50	0.46	261	232	7.0
0	Me			1.5	0	1	4	85	0.70	0.68	266	234	7.0
Experimental conditionf for the synthesis of 5'-methylphosphonates (III, R = Me)													
NH_2	H			1.5	0	6	12	52	0.42	0.40	270	247	7.0
											277	238	1.0
0	F			0.5	0	21	18	46	0.87	0.80	269	234	7.0

Table 3. Experimental conditions for the synthesis of 5'-methylphosphonates (I, R = Me)

X	Y	R^1	R^2	H1'	H2'	H3'	H4'	H5',5"	H-5	H-6	5Me	H-P
O	Me	H	F	6.11dt (6.1, 0.4)	5.6-5.0m		4.50m	4.05m		7.68s	1.89d (0.4)	6.79d (637.7)
O	Me	N_3	F	6.18t (6.2)	5.34dt (5.6, 5.1)		4.58m	4.14m		7.62s	1.87d (0.7)	6.29d (639.4)
O	Me	N_3	H	6.21t (6.6)	2.46t (6.2)	4.63t (5.45)	3.88s	4.20m		7.65d (0.5)	1.87d (0.8)	7.11d (637.5)
O	Me	F	H	6.21t (4.7)	4.56m	5.63t (8.2)	5.06t (8.2)	4.15t (4.9)		7.62d (1.1)	1.88d (0.8)	6.80d (639.7)
O	Me	H	H	6.40 (6.6)	2.3-2.1m		4.42m	4.06m		7.78d (1.1)	1.74d (1.1)	6.80d (636.3)
O	H	H	H	6.08dd (6.8, 6.0)	2.17m	3.36m	4.60m	4.02m	5.86d (6.6)	7.91d (6.6)		6.73d (637.5)
NH^2	H	H	H	6.4m	2.15m	3.52dd (10.7, 10.7)	4.20m	3.99m		8.04d (7.7)		6.71d (629.8)

H NMR parameters for 5'-hydrogenphosphonates in D_2O (II, R = J)

X	Y	R^1	R^2	H1'	H2'	H3'	H4'	H5',5"	H-5	H-6	5Me	H-P
NH_2	H			6.95t (5.0)	4.98m	6.0-4.5m	4.1-4.m	3.7-3.6m	6.45d (7.3)	7.72d (7.3)		6.48d (638.3)
O	Me			6.1-6.0m	4.5-4.3m	5.03t (10.7))	4.4-4.0m	3.62q (6.55)		7.98S		6.74d (637.7)

Table 3 (cont'd)

X	Y	R^1	R^2	H1'	H2'	H3'	H4'	H5',5"	H-5	H-6	5Me	H-P
1H NMR parameters for 5'-hydrogenphosphonates in D_2O (III, R = H)												
0	H			6.25m	4.5 ------------------- 4.8m					8.03d		6.78d
									(6.6)	(641.1)		
0	F			6.20s	5.9 ---------- 4.0m			4.3-4.2m		7.86d		6.75d
									(8.2)	(645.3)		
NH_2	H			6.20s	4.2 ------------------- 2.9m				6.08d (7.7)	7.85d (7.7)		7.14d (641.1)

*1 Chemical shifts in ppm (δ). signal description by apparent shape (e.g., t or q).

Coupling constants in Hz in ( ) right below chemical shifts first order.

For HP(0) $(OH)_2$, δ 6.88d (672.0 Hz).

Table 4. ^{2}H NMR parameters for 5'-methylphosphonates in D_2O (I, R=Me)

X Y	R^1	R^2	H1'	H2'	H3'	H4'	H5',5''	H5	H-6	5Me	Me-P
NH_2H	H	H	6.05m	1.78m	3.58d	4.12m	4.02m		8.58d		1.31d
					(10.7)			(7.7)		(16.5)	
O Me	H	H	6.25m	2.03m	3.12m	5.02m	4.0-3.8m	8.13s	1.41d	1.29d	
										(1.1)	(16.5)
O H	H	H	6.11t	2.13m	3.12m	5.02m	4.1-3.5m	5.86d	7.91d		1.27d
			(11.2)				(8.2)	(8.2)		(14.5)	
O Me	F	H	6.40t	4.53	5.72d	5.16m	3.7-2m		7.75s	1.93s	1.33d
			(8.8)	(4.1)							(16.2)
O Me	N_3	H	6.53t	2.80t	4.48m	3.88d	4.4-4.3m		7.99d	2.21d	1.22d
			(6.6)	(6.3)		(5.4)			(1.1)	(0.5)	(16.1
O Me	H	F	6.12t					7.68d	1.89d	1.36d	
			(6.1)					(1.2)	(1.2)	(13.2)	
O Me	N_3	F	6.52t	5.72t	4.78m	3.54t	4.5-4.4m		7.97d	2.23d	1.69d
			(4.6)	(8.4)		(7.40			(1.2)	(1.2)	(16.4)

^{1}H NMR parameters for 5'-methylphosphonates in D_2O (II, R=Me)

X Y			H1'	H2'	H3'	H4'	H5',5''	H5	H-6	5Me	Me-P
NH_2 H			6.93m	5.03d	5.98m	3.96m	3.88t	6.43d	7.79d		1.16d
				(5.0)			(10.4)	(8.5)	(8.5)		(16.5)
O Me			6.94m	4.53m	4.94t	3.98m	3.61m		6.59d	1.87d	1.16d
				(10.4)				(1.0)	(1.0)	(16.4)	
O H			6.81m	5.83m	6.50m	4.78m	4.01t	6.53d	7.30d		1.22d
							(5.5)	(8.0)	(8.0)		(16.5)

Table 4 (cont'd)

X Y	R[1]	R[2]	H1'	H2' H3'	H4'	H5',5''	H5	H-6	5Me	Me-P
[1]H NMR parameters for 5'-methylphosphonates in D_2O (III, R=Me)										
O F			6.25t	4.3-------	-------	-------3.2m		8.09d (6.6)		1.36d (16.5)
NH_2	H		6.19s	4.6-------	-------	-------3.5m	6.15d (8.5)	8.75d (8.5)		1.29d (16.5)

[1] Chemical shifts in ppm ( ). Signal description by apparent shape (e.g., t or q). Coupling constants in Hz in ( ) right below chemical shifts first order.

Table 5. ^{32}P NMR parameters for 5'-phosphonates in D_2O.

Structure	X	Y	R	R1	R2	Chemical shift ppm (δ)	Coupling constants (Hz)
I	NH_2	H	H	H	H	5.65m	929.9, 422.4,207.5
I	NH_2	H	Me	H	H	19.18m	541.4,302.2
I	O	Me	H	H	H	6.16d	637.0
I	O	H	Me	H	H	24.46m	551.7,305.0
I	NH_2	H	H	F	H	6.13d	634.4
I	NH_2	H	Me	F	H	5.59	619.5
I	O	Me	Me	N_3	H	6.29	637.2
I	O	Me	Me	F	H	26.72	636.6, 429.3, 214.6
III	NH_2	H	H			6.16m	634.3, 416.6, 208.1

Example 5

Anti-HIV-1 Assay. Anti-HIV-1 activities of the compounds were tested in MT4 cells. The cells were infected with HIV-1 at 200 TCID50 viruses per 10^6 cells. After an absorption period of one hour at 37°C unabsorbed viruses were removed by washing with fresh medium without fetal calf serum. The cells were suspended in fresh medium and distributed into 12-well microculture plates (10^6 cells 5/3ml/well). Then, various concentrations of test compounds were added. The cell cultures were incubated at 37° in a humidified atmosphere of 5% CO2 HIV-1 P24 core antigen and RT activity in the supernatants of the test cell cultures were detected on day-4. Anti-HIV-1 effects of compounds were evaluated by the inhibitory concentration was calculated by the median-effect plot using a computer software.

Cytotoxicity Assay. The Cytotoxicity of the compounds was determined in MT4 cells in 96-well microplates by XTT-microculture tetrazolium assay.

Table 7 lists the Anti-Hiv-1 effect and cytotoxicity of hydrogen-phosphates of pyrimidine nucleosides in MT4 cells.

Table 8 lists the Anti-Hiv-1 effect and cytotoxicity of hydrogen-phosphates of pyrimidine nucleosides in MT4 cells.

Table 9 lists the Anti-Hiv-1 Activity of AZT-HP, FLT-HP and ddt-HP based on Reversetranscriptase assay on day-4 in MT4 cells.

Table 10 lists the Dose-Effect relationships of inhibiting HIV-1 replication in MT4 cells.

Table 6. Results of initial screening of some nucleoside 5'-phosphonates in H9 cells using the indirect immunofluorescence assay together with EC_{56} obtained by the ELISA method and cytotoxicity IC_{50} data are summarized.

Compound*	Percent 100µM	Inhibition 10µM	1µM	Anti-HIV activity	EC_{50} *1 µM (MT4)	IC_{50} *2 mM (MT4)	IC_{50} *2 mM (MT4)	EC_{50}/IC_{50} *3 (MT4 cells)
AZT-HPO	80	50	0	++		>>5		
AZT-MePO	0	0	0	-				
FLT-HPO	99	98	80	++++	2.2	12.62	>>5	5,736
FLT-MePO	99	99	64	++++	0.30	4.60	5.5	15,333
LaFu-HPO	40	40	40	+		>>5		
LaFu-MePO	24	24	40	+		6.61		
LaC-HPO	100	70	40	+++		1.28		
LaC-MePO	99	99	40	+++		3.25		
ddC-HPO	40	40	0	+		2.32		
ddC-MePO	100	70	40	+++		7.44		
ddT-HPO	30	0	0	-		3.41		
ddt-MePO	10	40	40	+		9.32		
ddU-HPO	99	50	0	++	5.43	63.84	7.6	11,757
AdU-HPO	80	64	50	+++		>>1	28.9	
AdU-MePO	0	0	0	-		>>1	3.7	
ddU-MePO	80	50	24	++	2.7	3.58	25.0	1,326

Table 6 (cont'd)

Compound*	Percent Inhibition 100μM	10μM	1μM	Anti-HIV activity	EC_{50} *1 μM (MT4)	IC_{50} *2 mM (MT4)	IC_{50} *2 mM (MT4)	EC_{50}/IC_{50} *3 (MT4 cells)
d4C-HPO	99	70	40	+++		8.10		
d4C-MePO	40	24	40	+		>>5		
AZT	100	88	70		0.005	0.21	0.52	42,000
FLt	99	67	33		0.004	0.19	0.02	47,500
ddC	100	100	100		0.29	0.28	0.023	1,000

*HPO = 5-(H-phosphonate), MePO = 5'(Methylphosphonate)
AZT = 3'azido-3'-deoxythymidine
FLT = 3'-deoxythymidine
LaFU = 1-(2,30anhydro-ß-D-Lyxofuranosyl)-5-fluorouracil
LaC = 1-(2,3anhydro-ß-D0Lyxofuranosyl) cytosine
ddC = 2'3'-dideoxycytidine
ddT - 3'-deosythymidine
AdU = 3'-azido-2,3'-dideoxyuridine
ddU = 2,3'-dideosyuridine
d4C - 2',3'-dihehydro-2',3'-dideosycytidine (cytidinene)

*1 Concentration necessary to inhibit 50% of viral replication.
*2 Concentration required to inhibit 50% of cell growth.
*3 Therapeutic indeces.

Table 7. Anti-HIV-1 Effect and Cytotoxicity of 5'-Hydrogen-phosphonates of Pyrimidine Nucleosides in MT4 Cells

Compounds	Anti-HIV [a] EC50 (μM)	Anti-cell Growth [b] IC50 (μM)	IC50/EC50
AZT-HP	0.072	2,500	34,700
FLT-HP	0.135	>5,000	>37,000
ddT-HP	0.084	3,410	40,500
d4T-HP	11.98	>5,000	> 410
F-d4T-HP	> 50	>5,000	----
ddc-HP	3.91	2,320	590
d4c-HP	24.2	>5,000	> 210
LaC-HP	38.1	1,280	34
ddA-HP	> 50	>1,000	----
d4A-HP	12.25	>5,000	> 410
ddU-HP	5.34	>5,000	> 920
d4U-HP	51.0	2,300	45
AdU-HP	10.65	>5,000	> 470
LaU-HP	>100	>5,000	----
LaFU-HP	>100	>5,000	----
ddT	1.88	>5,000	> 2,660
ddC	0.29	2,280	7,860
ddA	5.04	1,493	300
AZT	0.005	154	30,800
FLT	0.004	190	47,500

a 50% effective concentration of inhibiting HIV-1 replication, based on P24-ELISA.

b 50% inhibitory concentration of MT4 cell growth, based on XTT-microculture tetrazolium assay.

Table 8. Anti-HIV-1 Effect and Cytotoxicity of 5'-Methyl-phosphates of Pyrimidine Nucleosides in MT4 cells

Compounds	Anti-HIV [a] EC50 (μM)	Anti-cell Growth [b] IC50 (μM)	IC50/EC50
AZT-MeP	133	>5,000	> 37
F-AZT-MeP	2.67	>5,000	>1,873
FLT-MeP	2.62	4,600	1,756
ddT-MeP	>100	>5,000	----
Fd4T-MeP	> 50	>5,000	----
ddc-MeP	10.03	>5,000	> 499
d4C-MeP	16.1	>5,000	> 310
LaC-MeP	>100	3,250	< 33
d4A-MeP	1.73	1,120	647
ddU-MeP	2.7	3,580	1,326
AdU-MeP	35	>5,000	> 143
LaFU-MeP	>100	>5,000	----
ddT	1.88	>5,000	>2,660
ddC	0.29	2,280	7,862
ddA	5.04	1,493	296
AZT	0.005	154	30,800
FLT	0.004	190	47,500

[a] See footnote a. in Table 1.

[b] See footnote b. in Table 1.

Table 9. Anti-HIV-1 Activity of AZT-HP, FLT-HP and ddT-HP based on RT assay on day 4 in MT4 cells

Compound	Anti-HIV EC50 (μM)[a]	Anti-cell Growth IC50 (μM)[b]	IC50/EC50
AZT-HP	0.2763	2,500	9,050
FLT-HP	0.1779	>5,000	>28,100
AZT	0.0139	154	11,079
FLT	0.0082	190	23,170
ddT	5.523	>5,000	> 900

a 50% effective concentration of inhibiting HIV-1 based on RT assay.

b 50% inhibitory concentration of MT4 cell growth based on XTT-microculture tetrazolium assay.

Table 10. Dose-Effect Relationships of Inhibiting HIV-1 Replication in MT4 Cells

Compound	(um)	% Inhibition		Median-effect Plot Parameters[a]		
		P-24 ELISA	RT Assay		P-24 ELISA	RT Assay
AZT:	0.1	99.20	97.89	Dm:	0.0087 ;	0.014
	0.05	97.39	92.14	m:	2.08 ;	1.98
	0.025	91.71	72.21	r:	0.994 ;	0.999
	0.0125	74.86	50.50			
	0.00625	37.76	15.02			
	0.00312	7.34	4.64			
AZT-HP	1.25	99.42	98.37	Dm:	0.072 ;	0.276
	0.625	97.90	94.18	m:	1.80 ;	3.17
	0.312	94.58	81.28	r:	0.994 ;	0.980
	0.156	73.99	13.96			
	0.078	57.13	1.0			
FLT-HP	1.25	99.17	98.13	Dm:	0.135 ;	0.177
	0.625	98.06	93.59	m:	2.36 ;	2.05
	0.312	88.38	72.94	r:	0.969 ;	0.999
	0.156	57.67	42.70			
	0.078	11.35	16.54			

[a] Dm (obtained from X-intercept) signifies the potentcy (the median-effect dose, e.g. ED50) m (slope) signifies the shape of the dose-effect curve (m=1,>1, and <1, indicate hyperbolic, signoidal, and negatively sigmoidal shapes, respectively; r (linear correlation co-efficient) determines the conformity of the dose-effect data to the median-effect principle of the mass-action law. All parameters are calculated by using a computer software for IBM-PC

EXPERIMENTAL DISCUSSION

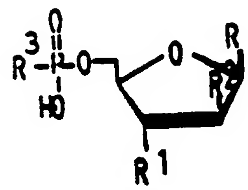
Representative compounds are tested for their inhibitory effects against human immunodeficiency virus in H-9 cells using 3'-azido-3'-deoxythymidine, 3'-deoxy-3'fluorothymidine and 2',3'-dideoxy-cytidine as standards. The results are summarized in Table 6. 1-(2,3-Dideoxy-5-O-[hydrogenphosphonyl]-ß-D-erythro-pentofuranosyl)uracil and 1-(2,3-dideoxy-2,3-didehydro-5-O-[hydrogen-phosphonyl]-ß-D-glycero-pentofuranosyl) thymine and inhibit replication of HIV at 10 micromolar concentration. Even at 1.0 micromolar concentration, these compounds inhibit HIV replication to a significant extent. The cytotoxicity of these compounds against uninfected cells is much less than that of the nucleosides used for standard.

The inventors have found that several compounds were found to be potent and selective inhibitors of HIV-1 replication. Among all of the active compounds, AZT-HP, FLT-HP and ddT-HP exhibited the most potent anti-HIV-1 activity. AZT-HP gave EC50 (50% antiviral effective concentration) of 0.072 μM and IC50 (50% inhibitory concentration of cell growth) of 2,500 μM. A selectively index of 34,700 was achieved. FLT-HP showed EC50 of 0.135 μM and IC50 of >5,000 μM. Its selectively index was >37,000. The EC50 and IC50 of ddT-HP were 0.084 μM and 3410 μM, respectively, with a selectivity index of 40,000. As control compounds, AZT, FLT and ddT gave their ED50, IC50 and selectively index as following: AZT, 0.005 μM, 154 μM and 30,800; FLT, 0.004 μM, 190 μM and 47,500; and ddT 1.88 μM and >5,000 μM and >2,660. Although AZT-HP and FLT-HP shows lower anti-HIV-1 activity than that of AZT and FLT, their selectivity indices were close to that of AZT and FLT. Their selectivity indices were close to that of AZT and FLT because of their low cytotoxicity. Anti-

viral activity of ddT-HP was more than 20-folds higher than ddT and it still shows low cytotoxicity. Thus ddT-HP gives a good selectivity index.

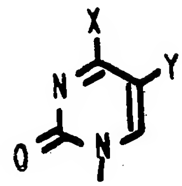
What is claimed is:

1. A compound having the structure:

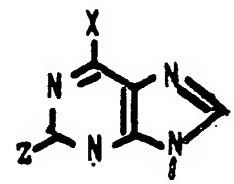


I

wherein R is



or



,

R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,

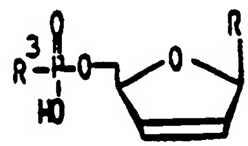
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

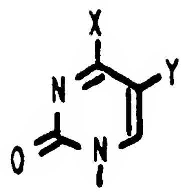
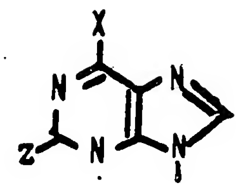
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

Z is a hydrogen, a hydroxy, or an amino group.

2. A compound having the structure:



II

wherein R is  or  ,

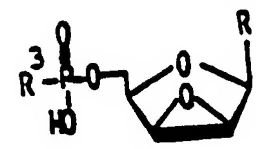
R^3 is hydrogen or an alkyl group of one to four carbons,

X is a hydroxy, a thiol, or an amino group,

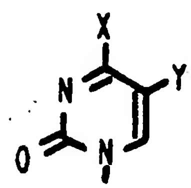
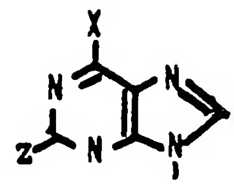
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and

Z is a hydrogen, a hydroxy, or an amino group.

3. A compound having the structure:

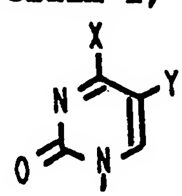


III

wherein R is  or  ,

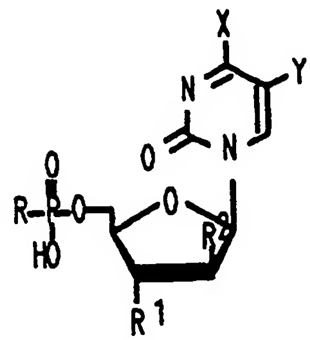
R^3 is hydrogen or an alkyl group of one to four carbons,
X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons, and
Z is a hydrogen, a hydroxy, or an amino group.

4. A compound of claim 1, 2 or 3 wherein R is:



wherein X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, halogen or an alkyl group of one to four carbons.

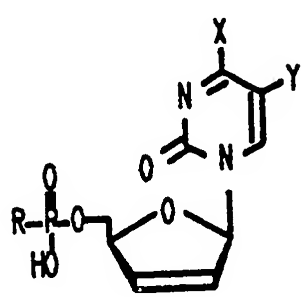
5. A compound having the structure:



I

X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

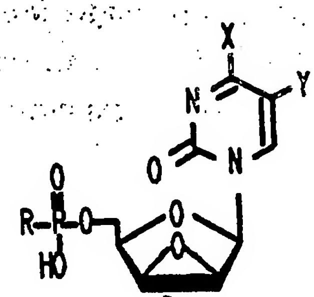
6. A compound having the structure:



II

X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

7. A compound having the structure:



III

X is a hydroxy, a thiol, or an amino group,
Y is hydrogen, a halogen or an alkyl group of one to four carbons,
R^1 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons,
R^2 is hydrogen, halogen, an azido, an amino or an alkyl group of one to four carbons, and
R is hydrogen or an alkyl group of one to four carbons.

8. A compound of claim 4 selected from the group consisting of:

1-(2,3-Dideoxy-5'-0-hydrogenphosphonyl-ß-D-glycero-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-0-hydrogenphosphonyl-ß-D-glycero-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-0-hydrogenphosphonyl-ß-D-glycero-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-0-hydrogenphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(3-Azido-2,3-dideoxy-5'-0-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-5'-0-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-5'-0-hydrogenphosphonyl-ß-D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-0-hydrogenphosphonyl-ß- D-erythro-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-0-hydrogenphosphonyl-ß- D-erythro-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-0-hydrogenphosphonyl-ß- D-erythro-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2-fluoro-5'-0-hydrogenphosphonyl-ß- D-threo-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2-fluoro-5'-0-hydrogenphosphonyl-ß- D-threo-pentofuranosyl) uracil,

1-(2,3-Dideoxy-2-fluoro-5'-0-hydrogenphosphonyl-ß- D-threo-pentofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl) cytosine, and

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-hydrogenphosphonyl-ß-D-arabinofuranosyl)-5-fluorouracil.

9. A compound of claim 4 selected from the group consisting of:

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-<u>glycero</u>-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-<u>glycero</u>-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-hydrogen-phosphonyl-ß-D-<u>glycero</u>-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) thymine, and

1-(2,3-Anhydro-5'-O-hydrogenphosphonyl-ß-D-lyxofuranosyl) uracil.

10. A compound of claim 4 selected from the group consisting of:

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-*glycero*-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-*glycero*-pentofuranosyl) thymine,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-*glycero*-pentofuranosyl) uracil,

1-(2,3-Dideoxy-5'-O-methylphosphonyl-ß-D-lyxofuranosyl)-5-fluorouracil,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-*erythro*-pentofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-*erythro*-pentofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-5'-O-methylphosphonyl-ß-D-*erythro*-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *erythro*-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *erythro*-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *erythro*-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *threo*-pentofuranosyl) thymine,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *threo*-pentofuranosyl) uracil,

1-(2,3-Dideoxy-3-fluoro-5'-O-methylphosphonyl-ß-D- *threo*-pentofuranosyl) cytosine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-ß-D-arabinofuranosyl) thymine,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-ß-D-arabinofuranosyl) uracil,

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-ß-D-arabinofuranosyl) cytosine, and

1-(3-Azido-2,3-dideoxy-2-fluoro-5'-O-methylphosphonyl-ß-D-arabinofuranosyl)-5-fluorouracil.

11. A compound of claim 4 selected from group consisting of:

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-ß-D-_glycero_-pentofuranosyl) cytosine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-ß-D-_glycero_-pentofuranosyl) thymine,

1-(2,3-Dideoxy-2,3-didehydro-5'-O-methylphosphonyl-ß-D-_glycero_-pentofuranosyl) uracil,

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) cytosine,

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) thymine, and

1-(2,3-Anhydro-5'-O-methylphosphonyl-ß-D-lyxofuranosyl) uracil.

12. A pharmaceutical composition which comprises a pharmaceutically effective amount of a compound of claims 1, 2, 3, 4, 5, 6 or 7 or pharmaceutically acceptable metal salt therof and a pharmaceutically acceptable carrier.

13. A method of treating a viral infection which comprises contacting the viral infection with an amount of the compound of claims 1, 2, 3, 4, 5, 6, or 7 effective to suppress viral replication.

14. A method of claim 13, wherein the infection is caused by human immunodeficiency virus.

15. A method of claim 13, wherein the viral infection is caused by heptitis virus.

16. A method of claim 13, wherein the viral infection is caused by cytomegalo virus.

17. A method of treating a subject afflicted with a viral infection which comprises administering to the subject an amount of the composition of claim 9 to effective suppress viral replication.

18. A method of claim 17, wherein the subject is a domestic animal.

19. A method of claim 17, wherein the subject is a human being.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04362

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): C07H 19/00

U.S.Cl: 536/23, 27, 28, 29; 514/49, 50

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/23, 27, 28, 29; 514/49,50

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Data bases searched: APS, CAS on line

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	US, A, 4,816,570 (FARQUHAR) 28 March 1989, see the entire document.	1-19
Y	Journal of Experimental Medicine, volume 166, issued October 1987, USA, D.R. Richman et al., "Failure of dideoxynocleosides to Inhibit Human Immunodeficiency Virus Replication in Cultured Human Macrophages," pages 1144-1149, see entire document.	1-19

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
05 September 1991	10 OCT 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Jeanne Marie for James O. Wilson

Form PCT/ISA/210 (second sheet) (Rev.11-87)